

Inhibitory Effect of *Nigella Sativa* Seeds and Curcumin on Hepatotoxicity Induced By Aflatoxin in Japanese Quails Associated With DNA Damage

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Abstract

This study is aimed to investigate the adverse effects of Aflatoxin (AFs) on performances, immunity and some biochemical parameters in serum and tissues of Japanese quails as well as to evaluate the possible protective effects of *Nigella sativa* (NS) crushed seeds and Curcumin powders to overcome the adverse effects of AFs. A total 120 unsexed Japanese quails chicks were divided equally into 4 groups; control group (G1), AFs group 2.5 ppm (G2), AFs 2.5 ppm plus *Nigella sativa* 1.5% (G3) and AFs 2.5 ppm plus Curcumin powders 400 mg/ kg diet (G4) for one month of experiment. AFs induced hepatotoxicity and immunosuppressive revealed by significant decrease in B.W, total leukocytes count and lymphocytes percent, while increase in heterophils percent, it also induced a significant decrease in serum total protein, albumin and globulin, on the other hand significant increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST), significant decrease in superoxide dismutase (SOD) and reduced glutathione (GSH) activity, while significant increase in malonaldehyde activity (MDA) and also in DNA fragmentation. Treatment with NS and Curcumin produce significant increase in B.W, TLC and lymphocytes percent, while decrease in heterophils percent, significant increase in total protein, albumin and globulin, on the other hand significant decrease in ALT and AST, significant increase in SOD, GSH activity and significant decrease in MDA activity and significance decrease in DNA fragmentation. This study indicated that treatment with *Nigella sativa* and Curcumin improved the adverse effects of AFs on performances, immunity, some biochemical parameters in serum and tissues and also gave more protection for DNA from fragmentation.

Key words: quails, AFs, *Nigella sativa*, Curcumin, immunity.

Introduction

Quails are considered very important that are used for eggs and meat (DAFF, 2013). The quail egg contains vitamins and antioxidants (Sahin et al., 2008), it has 3 to 4 times multi-nutritional content more than chicken eggs (Tunsaringkarn et al., 2013). Mycotoxins are poisonous substances produced by fungi, it's important to know that not every toxic substance synthesized by fungi is considered mycotoxin (Bennett and Klich, 2003). Mycotoxicosis is a current problem faces the poultry farmers (Madheswaran et al., 2004; Ortatatli et al., 2005). The factors affecting mycotoxin production are including fungal species potential, substrate composition, the duration of fungal growth, moisture percent, temperature degree, and storage environment. Stress factors such as shortage of water, insect infestation, and other pests' attack can also enhance toxin production (Sanchis and Magan 2004; Milani, 2013). Consumption of contaminated diet with AFs causes serious problems to animal's health because it has severed toxic, carcinogenic and mutagenic effects on it (Ferreira et al., 2013). Reduction of AFs in contaminated diet applies by many methods. Although, none of them has any value (Diaz et al., 2002). AFs makes significant changes in serum biochemistry parameters are generally regarded as indicative of

aflatoxicosis (Basmacioglu et al., 2005).

From the ancient times the medicinal plants and herbal remedies used in prevention and treatment of many diseases and disorders cure (Ashiq et al., 2014). *Nigella sativa* is an annual herbaceous plant; seeds are black in color and taste slightly bitter (Aljabre et al., 2005). The seeds of the plant are extensively used in traditional medicine in some countries, for the treatment of several diseases (Nostro et al., 2000).

NS seeds have many pharmacological properties as anti-carcinogenic (Rooney and Ryan, 2005), antiulcer (Kanter et al., 2005), antibacterial (Morsi, 2000), antifungal (Khan et al., 2003), anti-inflammatory, antipyretic and analgesic (Al-Ghamdi, 2001).

Curcumin structure (1, 7-Bis 4-hydroxy-3-methoxy phenyl-1-6-heptadiene-3, 5-dione) like diferuloylmethane was known Kazimierz Kostanecki, J. Miłobędzka and Wiktor Lampe (Shishodia et al., 2005).

The Aim of The present Work:

This study was aimed to investigate the effect of AF on Japanese quail as follows: (1) Preparation of AFs using standard toxigenic strain of *Aspergillus parasiticus*. (2) Study the effect of AFs on the body weight and performance of aflatoxicated Japanese quails. (3) Estimation of the immunosuppressive effect on the

cellular immunity. (4) Evaluation the effect of NS and Curcumin as anti-mycotoxin on Japanese quails treated with AF.

Materials and methods

Experimental birds and management:

A total number of 120, one-week-old apparent healthy unsexed Japanese quails obtained from Agricultural Technological Center, Faculty of Agriculture, Cairo University, Giza, Egypt. Chicks one-week-old were kept for one week for acclimatization at the Animal House of Veterinary Medicine then for 30 days of experiment. The chicks were randomly divided into four groups of average body weight (30.5g). Each treatment group contained 30 birds which were subdivided into three replicates, each of 10 chicks. According to (Hassan *et al.*, 2003), chicks were housed in wire battery cages of 86 L×50 W×25 H cm were equally partitioned into 3 pens (29×50×25cm). The batteries were provided with feeders and drinker equipments, the chicks were allowed *ad libitum* access to feed and water. Ventilation and temperature (22°C-31°C). Room were electrically heated and conditions of constant light were employed, provided 24 hours of lighting and checked three times daily for food, water and mortality.

Basal experimental diet:

Diet was formulated to meet the nutritional requirements as

suggested by the (NRC, 1994), to contain 24% CP and 2900 kcal ME/kg. Fresh feed were mixed weekly.

Standard toxigenic strains:

Toxigenic strain of *Aspergillus parasiticus* NRRL 2999 (ATCC) from Animal Health Research Institute Aldoki (AHRI) (Mycology department. **On Fresh potato dextrose agar medium (FPDA):** It used to stimulate spore formation of the mould strain, it was prepared according to *shotwell et al.*, (1966). Moldy rice was autoclaved, dried and ground to fine powder. AFs levels in rice powder were measured by HPLC method in the Mycotoxins Central Lab and Food Safety of the National Research Center. Milled rice was added to the basal diet to provide 2.5 mg AF /Kg diet (2500 ppb) according to *Eraslan et al.*, (2004).

Treatment

***Nigella sativa* seeds:** it was purchased from Isis company, registration No: COAE-Proc. /Exp. 111. COAE: Accreditation Certificate No.: DAKKs.ZE-3307.00. It was added freshly crushed with blender every week to the feed in a dose rate 1.5% according to *Tahan and Bayram*, (2011).

Curcumin powder (C₂₁H₂₀O₆). It was purchased from research lab company imported from India Batch No.557A110713. It was added to the diet in a dose rate 400 mg/kg diet according to *Tarasub et al.*, (2012).

Sampling:-

Every 10 days samples were taken from all groups by slaughtering 10 birds from each group for the measurement of cellular immunity, biochemical parameters and DNA fragmentation.

Blood sampling:- Two blood samples were collected in each slaughter; **the first** one was collected in EDTA tubes to prevent blood coagulation and used for determination of total leucocytic count according to the method described by *Natt and Herrick, (1952)*. And differential leucocytic count was performed using the method described by *Hoyer, (1993)*. **The second** blood sample was collected into a clean and dry screw capped centrifuge tubes without anticoagulant and left to clot at room temperature, then centrifugated at 3000 r.p.m for collection of clear serum. Sample used for the biochemical analysis of serum total protein, serum albumin, serum globulin and serum liver enzymes ALT and AST by commercial kits (Diamond, Egypt) according to methods described by *Young, (1995)* ; *Rodkey, (1965)* ; *Doumas and Biggs, (1972)* ; *Murray, (1984)*, respectively.

Tissues sampling:-

Biochemical analysis: - After the quails were slaughtered liver tissues were washed with normal saline then divided into 2 parts and preserved at -20° C. The first part for determination of Superoxide dismutase (SOD), Reduced glutathione (GSH), and Malondialdehyde (MDA) by commercial kits (Bio diagnostic, Egypt) according to methods described by *Nishikimi et al., (1972)* ; *Beutler et al., (1963)* ; *Satoh, (1978)* respectively, the second part of tissues were sent to Animal Health Research Institute (AHRI). Egypt. for determination of percentage of DNA fragmentation according to *Gibb et al., (1997)*. As in this formula:

$$\left[\frac{\text{OD(S)}}{\text{OD(S)} + \text{OD (P)}} \right] \times 100 = \text{\%Fragmented DNA}$$

Statistical analysis:

All the data were expressed as Means \pm S.E. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan's multiple range test (DMRT). Values were considered statistically significant when $p \leq 0.05$.

Table (A): the components of Basal experimental diet

Ingredient	Concentration (kg/100kg diet)
Ground yellow corn	55.780
Soya bean meal	31.960
Fish meal	1.000
Corn gluten	7.450
Bran	1.000
DicalciumPhosphate(22%Ca&19%P)	0.710
Limeston (38% Ca)	1.300
Lysine (purity 98%)	0.170
DL – Methionine (purity 98%)	0.070
Iodized sodium chloride	0.300
Mineral & Vitamin premix	0.300

Table (B): HPLC results by National Research Center: according to Nabney and Nesbit (1965)

Sample	AFs (mg/kg)				
	B1	B2	G1	G2	Total
AFs level in rice	4.65	0.02	0.08	0.06	4.81 ppm

Table (c): Experimental Design:

Group	No of quails	Age of quails/week				Time of experiment
			AFs 2.5 ppm	NS 1.5%	Curcumin 400mg/kg/diet	
G1	10	2	-	-	-	10 days
	10					20 days
	10					30 days
G2	10	2	+	-	-	10 days
	10					20 days
	10					30 days
G3	10	2	+	+	-	10 days
	10					20 days
	10					30 days
G4	10	2	+	-	+	10 days
	10					20 days
	10					30 days
Total	120					30 days

Table keys: + = treated - = non treated

G1: control -ve group **G2:** AFs group **G3:** AFs+ NS group **G4:** AFs + Curcumin group

Results:

Table (1) showed that G2 which received AF in a dose 2.5 ppm was significant ($P \leq 0.05$) decrease in body weight when compared to G1 after 10, 20 & 30 days, Also total leucocytic counts was significantly ($P \leq 0.05$) decreased lymphocyte percent, while heterophils percent was significantly ($P \leq 0.05$) increased. Table (1) showed that G3 and G4 were statistically significant ($P \leq 0.05$) increase in body weight when compared to G2 after 10, 20 & 30 days, G3 significantly increased TLC and lymphocyte percent, while decrease heterophils percent than G2, also G4 induced non significance changes in TLC and lymphocytes percent after 10 days but, showed significant ($P \leq 0.05$) increase when compared to G2 after 20 & 30 days, on the other hand G4 showed non significance changes after 20 days but, shows significant ($P \leq 0.05$) decrease heterophils percent at when compared to G2 after 10 & 30 days. Table (2) demonstrated that G2 was significant ($P \leq 0.05$) decrease in total protein, albumin and globulin when compared to G1 after 10, 20 & 30 days, while ALT and AST was significant ($P \leq 0.05$) increase when compared to G1 after 10, 20 & 30 days. Also Table (2) showed that G3 was significant ($P \leq 0.05$) increase in total protein and albumin

when compared to G2 after 10, 20 & 30 days. But G3 was non significance altered after 10 & 20 days although showed significant ($P \leq 0.05$) increase in globulin when compared to G2 after 30 days. However G3 had significant ($P \leq 0.05$) decrease in ALT and AST when compared to G2 after 10, 20 & 30 days. Moreover Table (2) showed that G4 was non significance changes in total protein and albumin after 10 & 30 days but, shows significant ($P \leq 0.05$) increase in total protein and albumin than G2 after 20 days, however G4 induced non significance changes in globulin when compared to G2 after 10, 20 & 30 days. However G4 was significant ($P \leq 0.05$) decrease in ALT when compared to G2 after 10, 20 & 30 days, while G4 had non significance changes after 10 days in AST but, shows significant ($P \leq 0.05$) decrease in AST when compared to G2 after 20 & 30 days. Table (3) demonstrated that the G2 was significant ($P \leq 0.05$) decrease in SOD and GSH in liver tissues when compared to G1 after 10, 20 & 30 days, However the G2 was significant ($P \leq 0.05$) increase in MDA activity in liver tissues homogenate when compared with G1. Table (3) showed that G3 and G4 were significant ($P \leq 0.05$) increase in SOD and GSH activity when compared to G2 after 10, 20

& 30 days, while that G3 and G4 were significant ($P \leq 0.05$) decrease in MDA when compared to G2 after 10, 20 & 30 days Table (3) & Fig. (1) showed that there were no significance changes in DNA fragmentation appeared in G1, G2, G3 and G4. DNA fragmentation

percentage when compared to G1 induced significant ($P \leq 0.05$) decrease in DNA fragmentation when compared to G2 after 20 & 30 days of experiment.

Table (1): Effect of dietary AFs on body weight changes (in grams), TLC, Lymphocytes percent and Heterophils percent of quails in control and experimental quails:

Groups		Control -ve (G1)	AFs (G2)	AFs+ NS (G3)	AFs+ Cur (G4)
Live body weight (g)	Initial	80.56±4.38 ^a	80.00±3.75 ^a	80.80±3.91 ^a	80.74±4.22 ^a
	Final	264.00±7.65 ^a	180.00±6.52 ^d	240.00±4.74 ^b	212.00±6.04 ^c
TLC (103 x mm ³)	10 days	24.60±0.92 ^a	17.20±1.50 ^b	21.60±0.81 ^a	18.00±0.71 ^b
	20 days	32.00±0.70 ^a	21.00±1.18 ^c	31.80±1.15 ^a	25.60±1.40 ^b
	30 days	35.40±1.80 ^a	18.20±2.00 ^c	36.20±1.20 ^a	27.00±0.83 ^b
Lymphocyte %	10 days	57.00±1.14 ^a	47.40±1.07 ^b	55.40±2.15 ^a	47.40±1.88 ^b
	20 days	58.00±1.26 ^a	47.60±0.74 ^c	57.80±0.86 ^a	51.20±0.85 ^b
	30 days	60.20±0.37 ^a	46.00±0.89 ^d	56.80±0.96 ^b	52.80±1.28 ^c
Heterophils %	10 days	34.40±1.22 ^c	42.20±0.86 ^a	35.00±0.71 ^{bc}	37.10±0.71 ^b
	20 days	35.18±1.32 ^b	46.80±0.97 ^a	36.40±0.93 ^b	43.20±1.66 ^a
	30 days	32.60±0.81 ^d	52.00±1.41 ^a	37.00±0.71 ^c	41.00±1.16 ^b

Values are means ± standard error (SE). Means within the same row with different superscripts are significantly different ($P \leq 0.05$).

Table (2): Effect of dietary AFs on [Tp, Albumin, Globulin, ALT and AST] in control and experimental quails:

Groups		Control -ve (G1)	AFs (G2)	AFs+ NS (G3)	AFs+ Cur (G4)
TP (g/dl)	10 days	3.17±0.15 ^a	2.06±0.01 ^c	2.50±0.20 ^b	2.26±0.09 ^{bc}
	20 days	4.50±0.16 ^a	3.23±0.06 ^c	3.89±0.22 ^b	3.70±0.08 ^b
	30 days	4.99±0.23 ^a	3.01±0.11 ^c	3.70±0.08 ^b	3.27±0.25 ^c
Albumin (g/dl)	10 days	1.74±0.06 ^a	1.22±0.08 ^b	1.67±0.05 ^a	1.18±0.92 ^b
	20 days	1.88±0.03 ^a	1.46±0.02 ^d	1.74±0.02 ^b	1.56±0.02 ^c
	30 days	2.05±0.05 ^a	1.53±0.15 ^c	1.90±0.06 ^{ab}	1.69±0.05 ^{bc}
Globulin (g/dl)	10 days	1.44±0.21 ^a	0.84±0.07 ^b	0.82±0.04 ^b	1.08±0.37 ^{ab}
	20 days	2.6±0.18 ^a	1.78±0.07 ^b	2.00±0.21 ^b	2.14±0.06 ^b
	30 days	2.94±0.23 ^a	1.48±0.21 ^b	2.40±0.08 ^a	1.58±0.24 ^b
ALT (U/l)	10 days	15.82±0.34 ^c	22.53±1.07 ^a	16.80±0.28 ^{bc}	18.31±0.97 ^b
	20 days	21.76±4.42 ^c	40.92±0.46 ^a	23.69±1.4 ^c	32.49±2.40 ^b
	30 days	18.84±0.56 ^c	65.80±5.43 ^a	33.98±1.83 ^b	41.81±0.81 ^b
AST (U/l)	10 days	165.95±2.08 ^b	187.69±2.64 ^a	169.23±3.18 ^b	181.58±2.78 ^a
	20 days	186.19±0.60 ^d	264.39±5.07 ^a	218.30±2.85 ^c	229.43±4.02 ^b
	30 days	193.06±2.03 ^d	320.16±5.25 ^a	230.01±1.19 ^c	241.76±3.65 ^b

Values are means ± standard error (SE).

Means within the same row with different superscripts are significantly different ($P \leq 0.05$).

Table (3): Effect of dietary AF on [tissue concentration of SOD activity, GSH activity, MDA and DNA fragmentation] of quails in control and experimental quails:

		Control -ve (G1)	AF (G2)	AF+ NS (G3)	AF+ Cur (G4)
SOD U/ g.tissue	10 days	12.25±0.26 ^a	7.5±0.08 ^d	10.26±0.08 ^b	8.92±0.16 ^c
	20 days	14.29±0.40 ^a	6.30±0.10 ^d	11.70±0.32 ^b	10.59±0.31 ^c
	30 days	15.38±0.24 ^a	4.36±0.48 ^d	13.27±0.47 ^b	11.59±0.24 ^c
GSH ng/g. tissue	10 days	4.65±0.07 ^a	1.71±0.12 ^d	3.49±0.12 ^b	2.85±0.17 ^c
	20 days	8.34±0.51 ^a	0.99±0.02 ^d	6.10±0.08 ^b	4.73±0.24 ^c
	30 days	8.88±0.15 ^a	0.91±0.03 ^d	7.59±0.39 ^b	5.64±0.11 ^c
MDA Mmol /g.tissue	10 days	27.49±1.06 ^c	58.25±5.46 ^a	33.87±1.6 ^{bc}	42.26±2.06 ^b
	20 days	35.62±1.25 ^d	109.31±3.11 ^a	61.49±3.17 ^c	86.13±3.9 ^b
	30 days	40.95±1.10 ^d	144.29±4.91 ^a	69.17±4.71 ^c	94.05±4.64 ^a
DNA fragmentation %	10 days	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
	20 days	4.23±0.03 ^d	19.77±0.63 ^a	7.37±0.60 ^c	11.13±0.16 ^b
	30 days	5.50±0.28 ^d	46.32±1.38 ^a	11.13±0.16 ^c	18.33±0.66 ^b

Values are means ± standard error (SE).

Means within the same row with different superscripts are significantly different ($P \leq 0.05$).

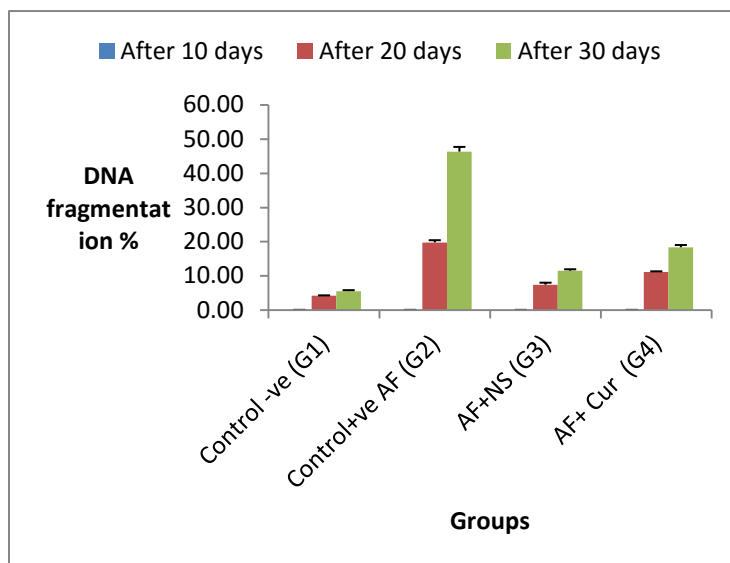


Fig. (1): Measurement of DNA fragmentation percentage in the liver tissues of quails.

Discussion

AFs eradication from animal diets and feed stuffs considers a major problem worldwide so there is a great need to create new technological techniques for its removal from diets (*Williams et al., 2004*). Serious diseases caused by AFs in animals lead to severe economic losses and death sometimes (*Mariam et al., 2013*). Many methods were used for detoxification of AFs include physical, chemical and biological methods. In our experiments we used medicinal plants like *Nigella sativa* and Curcumin for control of AFs.

Concerning this study, G2 was significant ($P \leq 0.05$) decrease in body weight at after 10, 20 & 30

days **Table (1)**, these results were in agreement with the findings of *Shehata et al., (2009)*. It might be due to that glutathione enzymes are utilized in process of detoxification to form methionine and cysteine, so the metabolic availability of methionine decrease and lead to poor feed efficiency and low growth rate (*Devegowda et al., 1998*).

According to our study which showed that G2 was significant ($P \leq 0.05$) decrease in total leucocytic count (TLC) and lymphocyte percent, while significant ($P \leq 0.05$) increase in heterophils percent after 10, 20 & 30 days **Table (1)**, these results come in accordance with results of *Mohapatra et al., (2011)*; *Selim et al., (2014)*. *Chamanza et al., (1999)* and *Bals, (2000)*.

Reported that the recruitment and migration mechanisms involved in cellular immunity are affected by AFs. Communication between cells of the immune system and innate responses frequently require different proteins in blood, depression of cellular immunity in AFs contaminated animals may be due to decrease of protein level in serum in affected poultry, especially the globulins level which antibodies are belonged (*Nazar et al., 2012*).

In the present study, G2 was significant ($P \leq 0.05$) decrease in total protein, albumin and globulin after 10, 20 & 30 days **Table (2)**, these finding is supported by *Rosa et al., (2001)* ; *El-Sayed and Khalil, (2009)* ; *Mohapatra et al., (2011)*. AFs adducts binds to macromolecules of liver cells which in turn causes alterations in protein synthesis and cellular integrity, also lead to decrease of serum total protein and albumin (*Jindal et al., 1994*; *Abo-Norag et al., 1995*). However, depletion of globulin is due to the toxicity of hemopoietic plus lymphocytolysis (*Sahoo et al., 2001*).

For the present study, G2 was significant ($P \leq 0.05$) increase in ALT and AST after 10, 20 & 30 days **Table (2)**, these results are in agreement with *Amiridumari et al., (2013)* ; *Selim et al., (2014)*. AST and ALT are present in the cytosol of the hepatocytes, their elevation in the blood stream means that AFs cause severe destruction inside the

hepatocytes and mitochondrial (*Mathuria and Verma, 2008*).

Concerning this study, the G2 was significant ($P \leq 0.05$) decrease in SOD, GSH activity in liver tissues after 10, 20 & 30 days **Table (3)**, these results correlate well with (*Mohamed et al., 2014*; *Naaz et al., 2007*; *Karabacak et al., 2015*), with significant increase in hepatic MDA activity in liver tissues homogenate when compared with control group **Table (3)**, these results are in accordance with (*Naaz et al., 2007*; *Wang et al., 2013*; *Eraslan et al., 2005*). During the metabolism of AFs by cytochrome p450 inside cells the reactive oxygen species (ROS) generate as super oxide anion, hydroxyl radical and H_2O_2 (*Josephy, 1997*; *Preetha et al., 2006*). SOD, an antioxidant enzyme is considered as the first and most important defense line against oxidative stress by catalyzing the conversion of superoxide anions or the active oxygen radical, produced in different stages of aerobic metabolism (*Yamakura and Fujimura, 1998*), to $2(O_2) + H_2O_2$ then catalase and glutathione peroxidase turn it into H_2O (*Afonso et al., 2007*). GSH plays an important role in the antioxidant system of the body. It maintains the normal structure and function of the cells via a redox and detoxification reaction (*El-Bahr, 2014*). *Eraslan et al., (2005)* explained that the decline in SOD enzyme activity observed upon administration of high doses of AFs may be related to

the consumption of highly active components during conversion into H_2O_2 due to the effect of AFs. That indicated the failure of antioxidant defense system to overcome the influx of ROS induced by AFs toxicity (*Piner and Üner, 2012*). Determination of MDA by thiobarbituric acid is used as an index of the extent of lipid peroxidation (*Andallu and Varadacharyulu, 2003*). The cytotoxic nature of AFs may be the underlying reason for the increase in MDA level (*Eraslan et al., 2005*). The significant reduction in the activities of antioxidant enzyme SOD and non-enzymatic antioxidant system (GSH) in aflatoxin treated group as compared to the control group could be responsible for increased TBARS levels observed during aflatoxin-induced hepatic damage (*Choudhary and Verma, 2005*). In this study, non-significant changes in DNA fragmentation appeared in G1, G2, G3 or G4 after 10 days of experiment **Table (3) and Fig. (1)**, in the other hand, G2 was significant ($P \leq 0.05$) increase in DNA fragmentation percentage than G1 after 20 & 30 days of experiment **Table (3) and Fig. (1)**, these results were also confirmed by (*Eshak et al., 2013*). AFs oxidase by the oxidase system to many hydroxylated metabolites and to AFBs 8, 9 epoxide that binds to mitochondrial DNA lead to formation of AFB1-DNA adducts which indicated its genotoxicity

(*Busby and Wogan, 1984*) and disturbs DNA replication causing genetic alteration (*Preston and Williams, 2005*). The favorable binding formation is with guanine lead to formation of AFB1-N-7 guanine adduct which is the cause of mutagenesis in AF quails (*Kallio and Lahdetie, 1997*). This interaction of AFs can be with the total genomic DNA (*Choy, 1993*). This can result in small to large changes in genomic DNA including micronuclei and DNA fragmentation (*Faridha et al., 2006*).

The result of these study showed that G3 and G4 were significant ($P \leq 0.05$) increase in body weight **Table (1)**, these results correlate well with other reports *Zaki et al., (2011); Sharma et al., (2011)*. *Lee et al., (2003)* reported that NS stimulate the enzymes of digestive as lipase and amylase beside mucosal secretion of intestine, these secretion are needed to enhance the digestion, to remove the pathogens adhesion and to establish the environmental media in intestine which the microorganisms balance are needed, so it improve the broilers performance by increasing the absorption and digestion of intestinal nutrients. Phenolic contents present in NS decrease the numbers of gut pathogens, so decreasing the loss of nutrients (*Nasir and Grashorn, 2010*). *Abdel-Wahhab and Aly, (2005)* Reported in NS groups showed improvement of all hematological

and biochemical parameters towards the normal values of the controls thus improve the body performance and thus increase the body weight. *Sharma et al., (2011)*, who explained that the co-administration with Curcumin improve bodyweight in comparison to AFB1 treated mice. It's suggested that antioxidant protection by Curcumin that is known to inhibit the biotransformation of AFB1 to aflatoxicol in liver (*Lee and Shibamoto, 2001*).

In the current study, G3 was significantly ($P \leq 0.05$) increased in total leucocytic counts and lymphocyte percent **Table (1)** also it significantly ($P \leq 0.05$) decreased in heterophils percent days **Table (1)**, these results are confirmed with *Abdel-Wahhab and Aly, (2005)* ; *Sharma et al., (2011)* and for G4 (*Asgary et al., 2012; Mahmoud, 2013*). NS prevents the AFs damage from its oxidation as the lipid peroxidation including in numerous diseases like cancer and immunosuppression diseases (*Lee and Shibamoto, 2001*).

From the present study, it is evident that Curcumin may stabilize the cell membrane and restore various blood variables. The lower and higher levels of Curcumin increased both erythrocyte and total leukocyte count. *Antony et al., (1999)* proved that Curcumin has immune-stimulating activity on animals by increasing the numbers of WBCs. The significance elevation in total protein, albumin and globulin in G3

Table (2) are supported with the other *Shehata et al., (2009)* ; *Ayoub et al., (2011)*. The inhibitory effect of *Nigella sativa* on AF production ability of *Aspergillus spp.* might be related to several components known to have biological activities, such as α -pinene and thymol and high phenolic content (*Maraqqa et al., 2007*), this finding might be due to the antioxidant effect of *Nigella sativa*. These observations are agreed with that of (*Al-Gaby, 1998*) who used *Nigella* cake protein as feed supplement. *Nigella sativa* crushed seeds (*Le et al., 2004*). The increase in plasma albumin and globulin which acts as an indicator of immune response and source of antibody from feeding broilers chicks rations contain 2.5% crushed NS may be attributed to the immune-stimulant effects of NS (*El-Ghammry et al., 2002*). The positive effect of Curcumin powder on serum values (total protein and albumin) **Table (2)** demonstrated their ameliorative effect against AFs, with Curcumin providing antioxidant protection decreasing the amount of AFB1 absorbed (*Gowda et al., 2008*). Biosynthesis and secretion of serum protein becomes normalized (*Mathuria and Verma, 2008*).

In the present work, the G3 was significantly ($P \leq 0.05$) decreased in ALT and AST **Table (2)**, these results are in agreement with *Ayoub et al., (2011)* ; *Nili-Ahmadabadi et al., (2011)*. The protective effects of

NS oil may be due to the radical scavenging activity of its components, whereas the protective effects of *S. aromaticum* oil may be due to the phenolic compounds present in the oil that decrease the formation of aflatoxin B1 epoxides by the inhibition of CYP450 enzymes and increase the ability of liver microsomes to catalyse aflatoxin–glutathione conjugation. Consequently, NS is quite useful and reasonable in the treatment of aflatoxicosis (*Abdel-Wahhab and Aly, 2005*). Concerning the present study, the positive results of Curcumin treated group G4 on decreasing ALT and AST, Similar results have been reported by (*Trivedi, 1999*). *El-Agamy, (2010)* Curcumin showed significant hepatoprotective activity by lowering the levels of serum marker enzymes ALT & AST. Treatment with Curcumin almost completely abolished the increase of serum ALT activity and decreased AST activity suggesting that Curcumin could effectively inhibit AFB1-induced liver cell injury.

the present study showed that G3 significantly ($P \leq 0.05$) increased SOD and GSH **Table (3)** and significantly ($P \leq 0.05$) decreased MDA **Table (3)**, these finding are in agreement with *Kanter et al., (2006)* ; *Nili-Ahmadabadi et al., (2011)*. Thymoquinone, has a strong antioxidant effect that inhibite lipid peroxidation, beside the presences of p-cymene, m-cymene, α -thujene and carvacrol as a consititeuent of

NS have also very powerful antioxidants activities and free radicals scavenging effects (*Burits and Bucar, 2000*). Current study show that G4 was significant increase in SOD activity and GSH activity **Table (3)**, these results are in agreement with *Mahmoud, (2013)*, and significant decrease in MDA **Table (3)**, these results are in agreement with *Naik et al., (2004)* ; *Mahmoud, (2013)*. Curcumin protects the antioxidant enzymes from denaturation as it has strong reactive oxygen species capacity (*Madkour, 2012*). *Masuda et al., (1999)* indicated that the antioxidant mechanism of curcumin still unknown, but it may be due to its reaction with glutathione also it turns to dimerization when reacted with free radicals.

G3 was significantly ($P \leq 0.05$) decreased in DNA fragmentation after 20 & 30 days of experiment **Table (3)** & **fig. (1)**, these results are confirmed by *El-Barbary, (2008)*. Where *Busby and Wogan, (1984)* reported how NS reduce the DNA fragmentation, that in the liver microsomes, AFB1 is oxidized to its reactive epoxide forming exo AFB-8, 9 epoxide. This subsequently links itself to DNA and exhibits the mutagenicity (*Lasky and Magder, 1997*). AFB1-DNA adduct disabilities the N-glycosidic bond of nucleotide leading to depurination and DNA strand scission (*Lyer et al., 1994*), So decreasing the binding formation to the AFB1, excision repair is the

system which primarily repair DNA-adducts (*Sancar and Sancer, 1988*). So this system may be activated via antioxidants which catalyse formation of polar, and conjugate between the epoxide intermediate of AFB and glutathione leading to reduce AFB1–DNA adduction (*Koob and Dekant, 1991*). G4 was significantly ($P \leq 0.05$) decreased in DNA fragmentation when compared to G2 after 10, 20 & 30 days of experiment **Table (3) & fig.(1)**, these results are confirmed by (*Siddique et al., 2010, Madkour, 2012*). DNA fragmentation observed in the present study is the normal consequence of oxidative stress that was demonstrated through elevation in LPO, and antioxidant enzymes (GPx, GST and GR) and glutathione content in rat liver (*Madkour, 2012*). This is also consistent with previous studies where DNA fragmentation was induced by AFs in rat lymphocytes (*Sharma et al., 2010*). G4 produced low DNA fragmentation, that ensures the supplementation of curcumin to AF quails reduce the DNA fragmentation these results coincide with that of (*Siddique et al., 2010*) who stated that Curcumin inhibits the generation of ROS that are responsible for the DNA damage. Also, this action of Curcumin was explained by (*Piwocka et al., 2001*) who stated that Curcumin led to attenuated

DNA fragmentation due to the normalization of GSH.

Conclusion:

Our results indicate that *Nigella sativa* crushed seeds and Curcumin powder have a protective effect against AF induced immunosuppression and hepatotoxicity in quails.

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الملخص العربي

التأثير التثبيطي لبذور حبة البركة و الكركم علي التسمم الكبدي المحدث بواسطة الأفلاتوكسن في السمان الياباني ومدى علاقته بتجزئة الذي أن أيه

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لقد هدفت هذه الدراسة الي الكشف عن الآثار العكسية للأفلاتوكسن علي الأداء، المناعة و بعض المعلمات البيوكيميائية في السيرم والأنسجة لدي السمان الياباني كذلك لتقييم التأثير الحمائي الممكن لبذور حبة البركة المطحونة و بودرة الكركم ضد التأثير العكسي للأفلاتوكسن. تم تقسيم اجمالي عدد ١٢٠ طائر من السمان الياباني غير محدد الجنس الي ٤ مجموعات ; المجموعة الضابطة (ج١)، مجموعة الافلاتوكسن ٢,٥ جزء من المليون (ج٢)، مجموعة الافلاتوكسن ٢,٥ جزء من المليون مع حبة البركة ١,٥% (ج٣) و مجموعة الافلاتوكسن ٢,٥ جزء من المليون مع بودرة الكركم ٤٠٠ ملجم/ كجم عليقة (ج٤) لمدة شهر واحد مدة التجربة. الافلاتوكسن أحدثت تسمم كبدي وتثبيط في المناعة يوضح ب نقص معنوي في وزن الجسم، عدد كرات الدم البيضاء الكلي و نسبة الخلايا الليمفاوية، بينما حدثت زيادة معنوية في نسبة الهيتيروفييل. الافلاتوكسن قام باحداث نقص معنوي في البروتين الكلي، الألبومين و الجلوبيولين، علي الجانب الأخر زيادة معنوية في الأئين أمينوترانسفيرس و أسبرتيد امينو ترانسفيرس، نقص معنوي في انزيم السوبرأكسيدديسموتيز، نشاط الجلوتاثيون المختزل ، زيادة معنوية في نشاط المالونداي الدهيد و نقص معنوي في نسبة تجزئة الذي.إن.إيه. أحدث العلاج بواسطة حبة البركة زيادة معنوية في وزن الجسم. عدد كرات الدم الكلي و نسبة الخلايا الليمفاوية، بينما أحدث نقص معنوي في نسبة الهيتيروفييل. حبو البركة والكركم أحدثت زيادة معنوية في البروتين الكلي، الألبومين و الجلوبيولين، علي الجانب الأخر نقص معنوي في الأئين أمينوترانسفيرس و أسبرتيد امينو ترانسفيرس، زيادة معنوية في انزيم السوبرأكسيدديسموتيز، نشاط الجلوتاثيون المختزل نقص معنوي في نشاط المالونداي الدهيد و زيادة معنوية في نسبة تجزئة الذي.إن.إيه. هذه الدراسة أوضحت أن العلاج بواسطة حبة البركة والكركم قد حسنت من التأثير العكسي للأفلاتوكسن علي الأداء، المناعة و بعض المعلمات البيوكيميائية علي السيرم والأنسجة وأيضا تعطي حماية أكثر للذي.أن.إيه من التجزئة.